

Ligation protocol

1. Set up the following reaction in a microcentrifuge tube on ice.
(T4 DNA Ligase should be added last. Note that the table shows a ligation using a molar ratio of 1:3* vector to insert.)

COMPONENT	Volume
10X T4 DNA Ligase Buffer	2 µl
Vector DNA (50 ng)	
Insert DNA (1:3 ratio)	
T4 DNA Ligase	1 µl
MB H2O	to 20 µl
Total	20 µl

2. Gently mix the reaction by pipetting up and down and microfuge briefly.
3. Incubate for 2 hr at 30 °C, or 16 °C overnight.

*How to calculate a molar ratio of 1:3

$$(insert\ amount[ng]) \times (plasmid\ length) = (plasmid\ amount[ng]) \times (insert\ length)$$
$$(insert\ amount[ng]) = \frac{(plasmid\ amount[ng]) \times (insert\ length)}{(plasmid\ length)} \times 3$$

Plasmid amount should always be 50 ng.